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PEPTIDE NUMBER	SEQUENCE	ANTISYNCTIAL POTENCY (μg/ml) IIIB	SF33	HIV-2	LAV-1
	428 445				
1005/45 (gp120)	C R I K Q F I N M W Q E V G K A M Y A P P I S G Q I R C	200 μg+	200 μg-	200 μg-	200 μg+
B26	R I K Q F I N M W Q E V G K A M Y A P P I S G Q I R C	200 μg+			
B119	C R I K Q F I N M W Q E V G K A M Y A P P	200 μg+			
1209/16	K Q F I N M W Q E V G K A M Y A P P I S G Q I R	440 μg+			
B138	K Q F I N M W Q E V G K A M Y A P P	40 μg+	40 μg+	20 μg+	40 μg+
1005/34	K Q I I N M W Q K V G K A M Y A P P	100 μg+	100 μg+	100 μg+	100 μg+
B92	K Q F I N I W L E W Y K A M Y A P P	400 μg-			
1029/04	R Q I I N T W H K V G K N V Y L P P	200 μg-	200 μg-	200 μg-	200 μg-
A24	W Q E V G K A M Y A P P	150 μg+			
A2A	K A M Y A P P	1 mg-			
	410 438				
1017/08 (gp120)	G D S T I T L P C R I K Q F I N M W Q E V G K A M Y A P P	400 μg+			
1005/33	G D S T I T L P C R I K Q F I N M W Q E	200 μg+			
	437 459				
1017/88 (gp120)	P P I S G Q I R C S S N I T G L L L T R D G G	200 μg+			
A5a	S N I T G L L L T R	300 μg-			
	473 485				
A27 (gp120)	F R P G G G D M R D N W R S E L	300 μg-			
A26	G D M R D N W R S E L	200 μg-			
1017/91 (Ateleria)	D I E K R I A K M E K A S S V F N V T M S	400 μg-			
	72 83				
1005/03 (MHC)	E E T V R F D S D V G E	400 μg-			
	38 65				
1005/78 (CD4)	C N Q G S F L T K G P S K L N D R A D S R R S L W D Q G	400 μg-			

(57) Abstract

Novel synthetic peptides and analogs thereof are provided that represent a neutralizing epitope in the external envelope glycoprotein (gp120) of the human immunodeficiency virus (HIV) that is purportedly involved in the binding of this molecule to its cellular receptor, CD4. Also provided are methods of generating antibodies reactive with or containing the internal image of the neutralizing epitope. The peptides or antibodies may be used in diagnostic immunoassays, as vaccines r in immunotherapy.

* See back of page

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NOVEL PEPTIDES ASSOCIATED WITH THE CD4 BINDING REGION OF GP120 AND THEIR METHODS OF USE

REFERENCE TO RELATED APPLICATIONS

The present application is a continuation in part of co-pending U.S. Patent Application No. 07/411,063, filed September 22, 1989.

FIELD OF THE INVENTION

The present invention relates generally to peptides and methods of selecting antibodies, more particularly, to novel peptides and analogs thereof associated with the CD4 binding domain or region of the gp120 segment of the HIV envelope protein and their use to generate HIV neutralizing antibodies for use in protective vaccination against AIDS or immunotherapy.

BACKGROUND OF THE INVENTION

As of June 1, 1989, 157,191 cases of AIDS had been reported to the World Health Organization (WHO). This is a conservative estimate of the extent of the AIDS epidemic; the WHO estimates that 5-10 million individuals are currently infected with HIV worldwide. Thus, the need for effective therapeutic and prophylactic reagents is paramount. Given the apparent failure of other vaccine candidates derived from the HIV envelope to protect primates against virus challenges, even in the presence of relatively high titers of neutralizing antibodies, it is apparent that these strategies employed to date are inadequate. It is probable that the problems associated with these potential immunomodulating agents were due to the quantitative and qualitative deficiencies in the neutralizing antibodies which they elicit. For example, such antibodies are frequently associated with reagents or epitopes on the envelope which are constantly mutating, a feature which enables the virus to stay a step ahead of the immune system. Perhaps the greatest challenge faced by researchers in the field of HIV vaccinology is currently identification of epitopes associated with gp120 molecule of HIV which will elicit broadly reactive or group-specific neutralizing responses which will afford protection against or substantially retard the progress of infection of the great majority if not all HIV types. Accordingly, the need exists for immunological surrogates to gp120 that represent a neutralizing epitope.

The human immunodeficiency virus ("HIV"), also formerly known as HTLV-III, LAV and ACV displays selective tropism for cells that express the CD4 receptor, primarily infecting the helper/inducer T cell lymphocytes. (See, Klatzmann, D., *et al.*, *Nature* ,312:767 (1984); Dalgleish, A.G., *et al.*, *Nature*, 312:763 (1984); Smith, D.H., *et al.*, *Science*, 238:1704 (1987); Fisher, R.A., *et al.*, *Nature*, 331:76 (1988).) This specificity for the CD4 receptor is mediated through the HIV envelope (*env*) glycoprotein gp120. Protective immune responses to the envelope protein of other retroviruses (Osterhaus, A., *et al.*, *J. Immunol.*, 135:591 (1985); and Earl, P.L., *et al.*, *Science*, 234:728 (1986)) as well as the biologically important roles of the HIV *env* protein in CD4 binding and syncytial formation (Lifson, J.D., *et al.*, *Nature* 323:725 (1986)) suggest that the conserved CD4 binding domain of gp120 is an

important target for developing strategies for controlling HIV infection (Weiss, R.A., *et al.*, *Nature*, 324:572 (1986)).

Proposed therapeutic strategies for HIV infection (including AIDS, ARC, etc.) that target the gp120/CD4 interaction include the *in vivo* administration to HIV positive patients of recombinant, soluble CD4 to compete with membrane-bound CD4 receptors for the HIV's gp120 (see, Clapham, P.R., *et al.*, *Nature*, 337:368 (1989) and Watanabe, M., *et al.*, *Nature*, 337:267 (1989)), the use of soluble CD4 coupled to toxic drugs to target infected cells (see, Chaudhary, V.K., *et al.*, *Nature*, 335:369 (1988)), and the use of anti-CD4 receptor antibodies as gp120 surrogates to boost or elicit an *in vivo* immune response ("active immunotherapy") that includes high titers of HIV neutralizing antibodies (see, McDougal, J.S., *et al.*, *J. Immunology*, 137:2937 (1986) and Chanh, T.C., *et al.*, *PNAS* USA 84:3891 (1987)). Prophylactic vaccination strategies also have focused on inducing anti-HIV, in particular, anti-gp120 antibodies.

Although these strategies are worthy of investigation, there are many inherent obstacles to successful therapy or vaccination. In particular, limitations on active immunotherapy and protective vaccination include the ability of the selected immunogen to elicit sufficiently high titers of HIV neutralizing antibodies and the ability of those anti-HIV antibodies to neutralize a broad group of HIV types or isolates. The numerous strains or types of HIV and its rapid mutation make the generation of antibodies that neutralize all of these variants ("group-specific") an essential element of effective immunotherapy or vaccination. This requirement is highlighted by the fact that native, recombinant, synthetic *vaccinia* encoded peptides from the HIV envelope has been used to immunize chimpanzees, but all have failed to protect these animals against infection. Thus, identification of a particular segment of gp120 that participates in the binding of CD4 and is also capable of eliciting a group-specific HIV-neutralizing immune response is a rational but elusive objective.

Unfortunately, the interactions of gp120 with CD4 at the molecular level and how these interactions affect viral tropism and receptor binding are not well characterized. Biochemical studies of gp120 have suggested that a putative CD4 binding site extends from amino acid residue 413 to

residue 456. (See, Kowalski, M., *et al.*, *Science* 237:1351 (1987) and Lasky, L.A., *et al.*, *Cell* 50:975 (1987).) The amino acid sequence numbering scheme for gp120 can vary depending on the HIV strain or isolate sequenced. For example, segment 397 to 439 discussed by Lasky is based on the $HTLV_{IIIB}$ strain and corresponds to 415 to 456 for the BRU strain. Anti-gp120 monoclonal antibodies, developed by immunization with the entire gp120, that block gp120 binding to CD4 have been mapped to residues 422-432; it has also been shown that a deletion mutant of gp120 involving residues 426-437 and a mutation at position 433 abrogates CD4 binding. (See, Lasky, L.A., *et al.*, *supra*.) Short peptide segments of the 415-456 region of gp120 have been suggested as T cell epitopes capable of eliciting cellular immunity, but these peptides have not been implicated as playing a role in humoral immunity. (See, Cease, K.B., *et al.*, *Proc. Natl. Acad. Sci.*, USA 84:4249 (1987).)

Although characterization of the 413-456 region of gp120 has been initiated at the one dimensional amino acid sequence level, it is important to appreciate that the neutralizing epitope of gp120 is a complex peptide conformation with primary, secondary and tertiary structure where amino acids interact both with each other to maintain the epitope's conformation and with the CD4 receptor to facilitate binding. Elucidation of the molecular basis for these structural and functional relationships in the native gp120 amino acid sequences and their immunological significance remains a considerable scientific challenge. Duplication of these structural and functional relationships of the neutralizing epitope is an essential feature of a gp120 surrogate and is the focus of the present invention.

Notwithstanding the designation of the "CD4 binding site" by Lasky, Kowalski, *et al.*, as being in the 413 to 456 range, applicants believe that the amino acid sequences on each side of the 413-456 region play a significant role in maintaining the immunologically active conformation of peptides mimicking the neutralizing epitope. Therefore, applicants have investigated the region from 335 to 517, which includes the CD4 binding region. The CD4 binding region may include the CD4 binding site identified by Lasky, Kowalski, *et al.*, but also incorporates additional amino acid sequences on the N and/or C terminus sides of this site.

SUMMARY OF THE INVENTION

The present invention is based on the identification of peptides and analogs thereof within the CD4 binding region of gp120 that contain one or more neutralizing epitope(s) involved in the binding of gp120 to its cellular receptor, CD4. More specifically, the present invention is based on peptides and immunologically equivalent analogs thereof from the gp120 region from about 335 to about 517 (amino acid position numbers may vary depending on HIV isolate).

In its broadest aspect, the present invention is directed to a compound characterized by the capability of eliciting and/or binding with HIV neutralizing antibodies, wherein the capability results from an amino acid sequence of at least 17 amino acid residues which: (a) is a neutralizing epitope(s) from the CD4 binding region of the gp120 envelope protein of an HIV isolate; (b) is a portion or segment of the epitope; or (c) is an immunological equivalent of the epitope or portion thereof.

The present invention further includes a method for generating antibodies for use as a vaccine or in immunotherapy. The first step of the method requires coating a solid support with an effective amount of a composition including the compound described above. Second, a serum sample containing polyclonal anti-gp120 or anti-compound antibodies is applied to the support, wherein a first plurality of the antibodies in the sample complex with the compound. The serum sample may be, for example, HIV positive human sera or sera from a mammalian host immunized with gp120 or a compound of the present invention. The solid support is then separated from the serum sample, and the first plurality of antibodies are eluted from the solid support. An immunogenic composition including the first plurality of antibodies is then formulated and used to immunize a mammalian host. A second plurality of polyclonal antibodies, which are immunologically reactive with the first plurality of antibodies and immunologically competitive with gp120, is selected and purified from the sera of the host. Alternatively, a second plurality of monoclonal antibodies is selected by sacrificing the host and generating hybridomas using an antibody producing organ of the host. The second plurality of monoclonal

antibodies are characterized as immunologically reactive with the first plurality of antibodies and immunologically competitive with gp120. This second plurality of antibodies are gp120 surrogates that may be used, for example, in the treatment or prevention of AIDS.

The present invention also includes a second method for generating antibodies for use as a vaccine or in immunotherapy. The first step of the method requires immunizing a first mammalian host with an immunogenic composition including a compound of the present invention. Then, a serum sample from the first host is applied to a solid support coated with gp120, whereby a first plurality of antibodies in the sample complex with the gp120. The solid support is separated from the serum sample, and the first plurality of antibodies eluted from the solid support. A second mammalian host is immunized with an immunogenic formulation including the first plurality of antibodies. A second plurality of polyclonal antibodies, which are immunologically reactive with the first plurality of antibodies and are immunologically competitive with gp120, is selected and purified from the sera of the second host. Alternatively, a second plurality of monoclonal antibodies may be selected by sacrificing the second host and generating hybridomas using an antibody producing organ of the second host. The second plurality of monoclonal antibodies are characterized as immunologically reactive with the first plurality of antibodies and immunologically competitive with gp120. This second plurality of antibodies are gp120 surrogates that may be used, for example, in the treatment or prevention of HIV infection.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts the sequences of examples of peptides within the scope of the present invention. Where tested, the various peptides' antisyncytial potentials for a number of HIV strains are also shown.

FIG. 2 shows flow cytometry assay results illustrating the ability of the B138 peptide to inhibit the binding of HIV I ($_{IIIB}$ and $_{RF}$ isolates) to CD4 positive T cells (CEM line). Also shown is the lack of inhibition by a control peptide 1005-05.

FIG. 3 shows proliferative responses of mononuclear cells from both HIV seronegative and seropositive individuals in response to peptide B138.

FIG. 4 shows further flow cytometric analysis depicting murine anti B-138 antibodies reacting with HIV infected T cells.

FIG. 5 shows ELISA assay results depicting the reactivity of sera from HIV seropositive and seronegative controls with peptide B138.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

As previously discussed, the conserved region of the CD4 binding region of gp120 is thought to be a potential target for controlling HIV infection. Studies to characterize those interactions between CD4 and gp120 that can stimulate or inhibit immune responses toward HIV are critical to the design of agents to treat HIV infections. Seeking to identify a neutralizing epitope(s) that plays a role in this gp120/CD4 interaction, the applicants examined some of the *in vitro* binding and immunological characteristics of peptides derived from the region of gp120 extending from amino acid residue 335 to 517 of various HIV isolates. (The amino acid position numbers may vary slightly from isolate to isolate.) This region of interest is referred to by the applicants as the "CD4 binding region."

Although applicants do not wish to be bound by any theory, peptide segments from this region were chosen based upon applicants' molecular conceptions of the topography of the CD4 binding site of gp120. Reasoning from studies on molecular mimicry of ligands by anti-receptor antibodies, applicants developed a model of the possible topography of the purported gp120 binding site for CD4 based upon a comparative modelling approach utilizing sequence homology with the immunoglobulin super family. Molecular modeling of the 415-456 region of gp120 from the BRU strain suggested to the applicants that a disulfide bond can be formed by the cysteine residues in this region of the molecule. Thus, applicants' model indicated that the residues 421-438 define a central turn region of the domain that is surface exposed for possible interaction with CD4. Based on this postulate, applicants undertook investigations of the 421-438 segment and analogs thereof that incorporate neutralizing epitope(s) of the CD4 binding region of gp120.

From this starting point, applicants' investigation was expanded to the study of equivalent CD4 binding regions of a number of HIV isolates--BRU, HXB2, MN, SK6W, SC, and CDC4. Based on infection data available to date, the MN isolate is the most prevalent strain of the virus in HIV infection and, therefore, peptides from this region are the most preferred. With the objective of mimicking the three-dimensional

conformation of the neutralizing epitope or epitopes within the CD4 binding region, applicants have designed numerous peptides incorporating amino acid sequences both upstream and downstream (toward the N terminus and C terminus) from B138 and 1005/45 peptides. In order to achieve favorable conformations and also to evaluate the effect of particular amino acid residues in the generation of HIV neutralizing antibodies, applicants have generated peptide analogs wherein specific amino acid residues have been substituted, deleted or added. Due to the complex folding nature of the peptides of gp120 in its native form, it is possible that amino acids remote from the actual "CD4 binding site" play significant roles in maintaining the conformation or structure of such an epitope. On the other hand, numerous amino acid residues and sequences thereof do not play a critical role (except to act as spacers) in maintaining a particular conformation or structure or in the binding of gp120 to CD4. Thus, substitution or deletion of such "spacer" amino acids may have no impact on a peptides' immunological properties. Thus, the present invention is not based on the precise delineation of amino acid sequences, but rather the present invention is based on the synthesis of peptides and compounds containing such peptides which share the immunological characteristic of being capable of eliciting and/or binding with the HIV neutralizing antibodies. The term HIV neutralizing as used in the present application includes both the ability to prevent HIV infection in new cells or the ability to block syncytial formation between cells.

Accordingly, in its broadest aspect, the present invention is directed to any compound which is capable of eliciting and/or binding with HIV neutralizing antibodies. This characteristic capability results from an amino acid sequence which is a neutralizing epitope(s) from the CD4 binding region of the gp120 envelope protein of an HIV isolate, or a portion or segment of such an epitope. Also, the amino acid sequence may be an immunological equivalent of the epitope or portion thereof. Alternatively, the present invention may include a compound, other than the naturally occurring HIV envelope protein, characterized by the capability of eliciting and/or binding with HIV neutralizing antibodies, wherein such compound includes the neutralizing epitope or segment thereof from the CD4 binding region of the gp120 envelope protein of an HIV isolate.

Based on applicants' investigations, the amino acid sequence of gp120 which participates in formation of the HIV neutralizing epitope stretches from at or about amino acid position 335 to at or about amino acid position 517, depending which HIV isolate is being analyzed. Although it may be preferred to generate peptide sequences and analogs from the MN isolate, it is preferred most that whatever isolate is used results in peptides that elicit HIV neutralizing antibodies that are grouped rather than type-specific. Although it is conceivable that type-specific peptides or neutralizing antibodies could be formulated in a cocktail for vaccine or immunotherapy use, it is preferred that a single peptide or HIV neutralizing antibodies elicited thereby be effective against all isolates and represent a conserved epitope from the CD4 binding region of gp120.

Also contemplated by the present invention are conjugates of the peptide or peptide analog that may enhance its immunogenicity. For example, the peptide or analog may be conjugated to such carrier proteins as tetanus toxoid, keyhole limpet hemocyanin (KLH), bovine serine albumin (BSA), and hepatitis B virus core antigen. The peptides of the present invention may also be conjugated to T cell epitopes designed to elicit a cellular immune response. Additionally, the peptides of the present invention may be conjugated to viral fusion proteins or included in chimeric constructs, *e.g.*, polio virus/peptide. Moreover, it is possible, using the basic methods reviewed by Reichmann, L., *et al.*, *Nature*, 332:323 (1988), for a skilled artisan to engineer a peptide of the present invention as an epitope loop into an entire antibody or fragment thereof, *e.g.*, chimeric antibodies.

As the peptide of the present invention represents an immunologically specific neutralizing epitope on the native gp120, it is imperative that the peptides, analogs, and conjugates thereof retain or be able to adopt the same three dimensional shape that contributes to the binding of CD4. Any derivative of the peptides of the present invention that meets this functional criteria is within the scope of the present invention.

Amino acids useful according to the present invention and their standard abbreviations are listed below in Table 1.

TABLE 1

ABBREVIATIONS FOR AMINO ACIDS

Amino Acid	Three-letter abbreviation	One-letter symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Asparagine or aspartic acid	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glutamine or Glutamic acid	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tyrosine	Tyr	Y
Valine	Val	V

The compounds of the present invention typically include a peptide of at least 17 to about 100 amino acid residues. The peptide may be selected from any one of the amino acid sequences listed in Table 2. Alternatively, the peptide may be a peptide analog of the amino acid sequences listed in Table 2 where the amino acid residues are modified, substituted, deleted, inserted or added to the sequence, provided that

the immunoreactivity of the analog to anti-peptide antibodies (which are also reactive with gp120) is preserved in the peptide analog.

TABLE 2

ANALOGS OF B138

PEPTIDE	SEQUENCE	SOURCE
1029/16 (BRU Isolate):	KQFINMWQEVGKAMYAPPISGQIR	B138
1029/76 (BRU Isolate):	KQFINIWLEVGKAMYAPP	B138
1029/77 (BRU Isolate):	KQFINTWLHEVGKAMYAPP	B138
1029/81 (BRU Isolate):	KQFINMWLEVGKAMYAPP	B138
1029/82 (BRU Isolate):	KQFINIWQEVGKAMYAPP	B138
1029/83 (BRU Isolate):	KQFINMWREVGKAMYAPP	B138
1029/84 (BRU Isolate):	KQFINTWQEVGKAMYAPP	B138
1029/85 (BRU Isolate):	KQFINMWQEVGKNVYAPP	B138

1029/86 (BRU Isolate):

KQFINMWQEVGKAVYAPP B138

1005/28 (BRU Isolate):

KQFINMWQE B138

1005/33 (BRU Isolate):

GDSTITLPCRIKQFINMWQE B138

1017/08 (BRU Isolate):

GDSTITLPCRIKQFINMWQEVGKAMYAPP B138

1017/88 (BRU Isolate):

PPISGQIRCSSNITGLLLTRDGG C-TERM GP120

1005/34 (HXB2 Isolate):

KQIINMWQKVGKAMYAPP B138

1005/70 (HXB2 Isolate):

GDSTITLPCRIKQIINMWQK B138

1005/29 (HXB2 Isolate):

KQIINM•QKVGKAMYAPP B138

1139/43 (MN Isolate):

KQIINMWQEVGKAMYAPP MN

1029/04 (SK6W Isolate)

RQIINTWHKVGKNVYLPP SK6W

CYCLIC ANALOGS OF 1005/45

PEPTIDE	SEQUENCE	SOURCE
1043/68 (SC Isolate):	C*RIKEIINMWQEVGKAMYAPPIKGQVKC*	1005/45
1043/72 (CDC4 Isolate):	C*RIKQIINRWQVVGKAMYALPIKGLIRC*	1005/45
1077/36 (CDC4 Isolate):	C*HIRQIINTWHKVGKNVYLPPREGDLTC*	1005/45

ADDITIONAL PEPTIDES FROM THE CD4 BINDING SITE

PEPTIDE	SEQUENCE
1103/01 (BRU Isolate):	KQIINMWQEVGKAMYAPPIEGQIR
1103/02 ("T1" region plus BRU Isolate):	QMHEDIISLWDQSLKKQIINMWQEVGKAMYAPPIEGQIR
1103/68 (MN Isolate):	C*GGEFFYGGKIKQIINMWQEVGKAMYAPPIEGQIRC*
1139/35 (MN Isolate):	SSNITGLLLTRDGGKDTDTNDTEIFRPGGGDMRDNWRS
1139/60 (MN Isolate):	NKTIVFNQSSGGDPEIVMHSFNC*GGEFFYG•GKIKQIINMWQE VGKAMYAPPIEGQIRC*SSNITGLLLTRDGGKDTDTNDTEIFRPG GGDMRDNWRS

1139/64 (MN Isolate):
KQIINMWQEVGKAC*YAPPIEGQIRC*

1139/65 (MN Isolate):
KQIINMWQEVGKAC*YAPPIEGQIRC*SSNITGLLLTRDGG

1139/63 (MN Isolate):
FYCNTSPQLFNSTWNGNNTWNNTTGSNNNITLQCKIKQIIN
MWQEVGKAMYAPPIEGQIRCSSNITGLLLTRDGG

Notes to Table 2 Sequence Information:

-- Underlining indicates a substitution of a new amino acid residue for the amino acid residue of the naturally occurring sequence.

-- The symbol • indicates that a naturally occurring amino acid residue(s) has been deleted.

-- The symbol * associated with a cysteine ("C") residue indicates that a disulfide bond may be formed between two such cysteines.

The amino acid cysteine ("C") is highlighted by an asterisk ("*") to denote its special function. In addition to forming a site for cross linking to carrier proteins, if a pair of cysteine residues are sufficiently far apart, they are capable of forming a disulfide bond between one another and thereby contributing a loop-like configuration to an amino acid sequence. Thus, the three dimensional conformation of peptide sequences can be modified by the addition or deletion of cysteine residues. For example, in the 1139/60 peptide, a single loop bracketed by two amino acid sequences is formed by a disulfide bond between the two C* with the amino acid sequences outside the loop (termed "tails") contributing to the conformational or binding properties necessary to mimic the neutralizing epitope. It is also notable in the 1139/60 peptide that two glycines have been substituted for the native cysteines and a substantial amino acid sequence has been deleted from the peptide. This deleted amino acid sequence represents a known hypervariable domain within the CD4 binding region that may potentially interfere or with at least not contribute to a peptide's capability of eliciting group-specific HIV neutralizing antibodies.

A skilled artisan may synthesize any of the peptides of the present invention by using an automated peptide synthesizer (for example, an Applied Biosystems model 430A) using standard t-Boc chemistry as reported by Carpino, L.A., *J. Am Chem Soc*, 79:4427 (1957), the disclosure of which is hereby incorporated by reference.

Analogs of the preferred peptides can be prepared by varying the number, order, or type of amino acids used in the standard synthesis method. The term "analog" as used herein encompassed any of the peptides in the present invention, segments thereof, peptides that contain substitutions or modifications of amino acid residues, and peptides that contain insertions or deletions of amino acids in the peptide or additions to the terminal amino acid residues of the peptide.

Additional examples of peptide analogs are shown in FIG. 1 and include:

KQFINMWQEVGKAMYAPPISGQIR, ("1029/16")

wherein S is Serine and R is Arginine; and

CRIKQFINMWQEVGKAMYAPPISGQIRC, ("1005/45")

wherein C is Cysteine. The peptide designated 1005/45, although depicted as a linear amino acid sequence above, is preferably cyclized by a disulfide bond between the terminal cysteines. All data, except the coupling of the peptide to the affinity column, that refers to the 1005/45 peptide was developed with the cyclic form. Although applicants do not wish to be bound by any theory, it is postulated that the cyclic form of 1005/45 is a more rigid structure that locks in the conformational shape of the epitope more effectively than a linear peptide, *e.g.*, B138.

Table 3 summarizes applicants' evaluation the biological properties of two of the peptides of the present invention, B138 and 1005/45.

TABLE 3

SUMMARY OF THE BIOLOGICAL PROPERTIES OF THE HIV ENVELOPE-DERIVED SYNTHETIC PEPTIDES B138 AND 1005/45

	B138	1005/45
Peptide structure	*env* residues 421-438 linear form	*env* residues 418-445 cyclized via disulfide bonds on terminal cysteines
Syncytial blocking	40 µg/ml	200 µg/ml
Antisera (rabbit)		
Anti-peptide titer	> 1:100,000*	> 1:100,000*†
Anti-gp120 titer	> 1:50,000*	> 1:50,000*†
Multi-strain reactivity (FACS)	Binds to $HTLV_{IIIB}$, LAV and SF33	Binds to $HTLV_{IIIB}$, LAV and SF33
Radioimmuno-precipitation	Reacts strongly with gp120. Soluble CD4 partially inhibits reaction	Reacts strongly with gp120. Soluble CD4 partially inhibits reaction
CD4-gp120 inhibition assay	Inhibits ~50% of gp120-CD4 reaction	Inhibits ~50% of gp120-CD4 reaction

* Sera obtained from hyperimmunized animals (after receiving six inoculations of peptide [100 mg] in SAF-m).

† Although in some cases the responses elicited by B138 equalled those achieved by 1005/45, the cyclic peptide was generally the more efficient immunogen and induced antibody titers approximately ten-fold greater than B138.

Peptides of the present invention are designed to contain a neutralizing epitope of gp120 and, therefore, may be used as gp120 surrogates in targeting the gp120/CD4 interaction. For example, the peptide may be

used as a vaccine in uninfected individuals to induce protective titers of neutralizing anti-HIV antibodies. Alternatively, the peptide may be used in active immunotherapy of HIV positive individuals to initiate or boost their immune response of neutralizing anti-HIV antibodies. Moreover, the peptides of the present invention may be immunosuppressive due to their interaction with CD4 receptors and, therefore, may be useful in modulating the immune response in autoimmune diseases.

Additionally, the peptides of the present invention may also be used to generate antibodies that can be used as templates to generate anti-idiotype antibodies having the internal image of the neutralizing epitope structure contained in the peptide sequence. These antibodies, polyclonal or monoclonal, can then be used in vaccine formulations or in active immunotherapy. Accordingly, the present invention also includes monoclonal or polyclonal antibodies that carry the internal image of the peptides, as well as methods for generating these antibodies. Like the peptides *per se*, these antibodies may be used as gp120 surrogates.

A first method for generating such antibodies for use as vaccines or in active immunotherapy starts with coating a solid support with an effective amount of a peptide of the present invention. The process of coating or linking a peptide to a solid phase support potentially alters the peptides' three-dimensional conformation, therefore it may be necessary to explore several techniques in order to achieve the desired technical results. A preferred solid support is Reacti-Gel™ obtained from Pierce Biochemicals, Rockford, IL. This support matrix comprises a cross-linked agarose gel that is activated by carbonyldiimadazole (CDI). The imidazoyl-carbamate group couples to ligands containing free amino groups most efficiently at a pH of between 9-11, although sensitive proteins can be coupled at pH 8.5 in 0.1M borate buffer. Alternative techniques include the use of cyanogen bromide (CNBr)-activated agarose which also couples peptides or proteins via free amino groups (Hudson, L. and Hay, F.C., *Practical Immunology*, 3rd edition, Blackwell Scientific Publications, Oxford, 1989).

Once the coated solid support is prepared, a serum (as used herein "serum" is intended to included both serum and plasma) sample

containing anti-gp120 antibodies is then applied. Suitable serum samples include, for example, human serum from HIV positive individuals, or sera from a mammalian host immunized with gp120 where these sera can be shown by ELISA to be reactive with one or more of the peptides of the present invention, or with a peptide composition of the present invention. A subset of the anti-gp120 or anti-peptide antibodies present in the serum complex with the support bound peptide. The support can then be separated from the serum sample and the complexed anti-gp120 or anti-peptide antibodies eluted from the support. Eluting agents useful according to the present method include acidic buffers such as 0.1M glycine-HCl, pH 2.5 to 4.0.

This first plurality of anti-gp120/anti-peptide antibodies is formulated in a immunogenic composition and used to immunize a mammalian host, preferably a rabbit or guinea pig. The host is bled and a second plurality of polyclonal antibodies is then selected and purified, *e.g.*, over an affinity column coated with the immunogen, from the sera of the host. Preferably, a second plurality of monoclonal antibodies may be produced by using the basic method developed by Köhler and Milstein, reported in *Nature*, 256:495-97 (1975), the disclosure of which is hereby incorporated by reference. Briefly, this procedure would include: sacrificing the mammalian host immunized with the first plurality of antibodies; harvesting an antibody producing organ, *e.g.*, spleen, from the host; preparing a cellular homogenate from the harvested organ; fusing the cellular homogenate with a malignantly transformed cell line, *e.g.*, myeloma cells; selecting or screening for hybrid cells that produce monoclonal antibodies specific for the first plurality of antibodies and capable of competing immunologically with gp120; cloning the hybrid cells, i.e., hybridomas, so that they can produce perpetually; and harvesting monoclonal antibodies that are produced by the selected hybridomas. Whether polyclonal or monoclonal, this second plurality of antibodies is characterized by their immunological reactivity with the first plurality of antibodies and immunological competition with gp120. Additionally, the second plurality of antibodies may be characterized by their ability to induce in mammals titers of neutralizing anti-HIV antibodies. The second plurality of antibodies contain the internal image of the neutralizing epitope and, therefore, are candidates for use as a vaccine or in immunotherapy.

A second method for generating such antibodies starts with the immunization of a mammal host, preferably a rabbit or guinea pig, with a peptide composition of the present invention. Where the peptide is not sufficiently immunogenic in the selected host, it can be formulated with an adjuvant and/or, like other haptens, an immunogenic response achieved by linking the peptide to a carrier. Suitable adjuvants include muramyl dipetide adjuvants, *e.g.*, SAF (Syntex Adjuvant Formulation), Freund's adjuvant, and alum. Methods of linking carriers to haptens are well known in the art; and numerous carrier proteins are available for coupling with the peptides, *e.g.*, KLH and tetanus toxoid. A serum sample from the host is then applied to a solid support coated with gp120, whereby a first plurality of antibodies in the sample complex with the support bound gp120. The gp120 isolate that is selected is not critical. However, if a particular HIV isolate is prevalent in the patient population targeted for treatment, that gp120 isolate may be selected for use on the solid support, so that the antibodies selected by the method are tailored for the HIV epidemiology. The support is separated from the serum sample and the complexed antibodies eluted from the gp120 coated support. This first plurality of antibodies is then used to immunize a second mammal host, preferably a mouse. A second plurality of polyclonal antibodies may be selected and purified, *e.g.*, using an affinity column, from the sera of the second host. Preferably, a second plurality monoclonal antibodies is selected by sacrificing the second host and generating monoclonal antibody producing hybridomas according to the procedure described above. This second plurality of antibodies, whether polyclonal or monoclonal, is characterized by their immunological reactivity with the first plurality of antibodies and their immunological competition with gp120. Additionally, the second plurality of antibodies may be characterized by their ability to induce in mammals titers of neutralizing anti-HIV antibodies. The second plurality of antibodies contains the internal image of the neutralizing epitope represented by the peptide, and, therefore, are candidates for use as a vaccine or in immunotherapy.

A third method for generating antibody surrogates for gp120 starts with the immunization of a first mammalian host with an immunogenic composition including a peptide composition of the present invention.

Then, using the procedure previously described, a hybridoma is generated from an antibody producing organ of the first mammalian host, where the hybridoma is capable of producing a monoclonal antibody characterized by its immunological reactivity with the peptide immunogen and its immunological reactivity with gp120. Additionally, this first monoclonal antibody may be characterized by its ability to neutralize HIV infection of CD4 positive T cells *in vitro*. Then, a second mammalian host is immunized with an immunogenic composition including the first monoclonal antibody. A plurality of polyclonal antibodies may be selected and purified from the sera of the second host, wherein the polyclonal antibodies are characterized by their immunological reactivity with the first monoclonal antibody and immunological competition with gp120. Additionally, the second plurality of antibodies may be characterized by their ability to induce in mammals titers of neutralizing anti-HIV antibodies. Preferably, monoclonal antibodies are produced by generating a second hybridoma from an antibody producing organ of the second mammalian host. The hybridoma is capable of producing a second monoclonal antibody characterized by its immunological reactivity with the first monoclonal antibody and immunological competition with gp120. Additionally, this second monoclonal antibody may be screened for its ability to block syncytia of HIV *in vitro*. The monoclonal and polyclonal antibodies produced by this method also contain an internal image of the neutralizing epitope represented by the peptide, and, therefore, are candidates for use as a vaccine or immunotherapy.

An additional aspect of the present invention is the generation of monoclonal antibodies to the peptides of the invention. Using the basic methods developed by Köhler and Milstein, discussed above, a skilled artisan may develop hybridoma cell lines producing monoclonal antibodies specific for the peptides of the present invention. In contrast to the monoclonal antibodies previously discussed, these monoclonal antibodies would not contain an internal image of the neutralizing epitope but rather would contain the mirror image of the neutralizing epitope and, therefore, specifically bind to such epitope. The murine monoclonals could be "humanized" by the some of the techniques described by Reichmann, L., *et al.*, *Nature*, 332:(6162) 323 (1988) the disclosure of which is hereby incorporated by reference. Alternatively,

human monoclonals could be made by a variety of methods, including the EVB transformation of PBL's from HIV positive individuals who's sera can be shown by ELISA to be reactive with one of the peptides of the present invention. (See, Banapour, B., *et al.*, *J. Immunol.*, 139:4027 (1987), the disclosure of which is hereby incorporated by reference.) In any event, these murine or human monoclonal antibodies will bind specifically to the neutralizing epitope of gp120 and, thus, are suitable *per se*, or for linking to toxins or anti-viral reagents for targeting of HIV, in passive immunotherapy.

A further aspect of the present invention is the use of the peptide of the present invention in immunoassay methods for the detection of anti-HIV antibodies for the diagnosis, prognosis, or therapeutic monitoring of AIDS, ARC or pre-AIDS conditions. Such immunoassay methods include coating a solid support with an effective amount of a peptide of the present invention, which functions as an antigen. A test sera diluted with buffers is then applied to the solid support, where anti-HIV antibodies in the test sera form peptide antibody complexes with the support bound peptide. The mixture is then incubated at room temperature and the presence of the peptide/antibody complex is detected. Such immunoassays are preferably enzyme linked immunoassays ("ELISA") where a second known anti-human antibody tracer, capable of complexing with the anti-HIV antibody, is labeled with an enzyme and applied to the mixture forming a peptide-antibody-antibody sandwich. The second known antibody is, for example, goat anti-human IgG antibody labeled with horseradish peroxidase. The sandwich is detected by adding a substrate for the enzyme to form a colored product. A suitable substrate for horseradish peroxidase is a mixture about 0.04% by weight orthophenylenediamine and about 0.012% by volume hydrogen peroxide in sodium citrate buffer, pH 5.0. An alternative embodiment is an immunoradiometric assay ("IRMA"), which utilizes a second known anti-human antibody labeled with a radioactive element, *e.g.*, ^{125}I-labeled. Further, the peptide antibody complex may be detected as an agglutination in an agglutination assay. Such assays may be manufactured and sold as test kits which would include: a solid support; an immunoadsorbant including a peptide composition of the present invention coated on the solid support; a sample of normal serum as a negative control; a sample

of serum containing anti-HIV antibodies as a positive control; and buffers for diluting the serum samples. The enzyme labeled anti-human antibody or radiolabeled anti-human antibody may be also included in the kit.

EXAMPLE 1: SYNTHESIS, PURIFICATION AND CHARACTERIZATION OF PEPTIDES

Synthesis

Peptides were synthesized on an Applied Biosystems 430A automated peptide synthesizer using pMBHA resin as a solid support (Matsueda, G.R., *et al.*, *Peptides*, 2:45 (1981)). All amino acids were coupled by pre-formed symmetric anhydrides (Yamashiro & Li, *J. Am Chem. Soc.*, 100:5174 (1978)) with the exception of asparagine and glutamine which were coupled as their hydroxybenzotriazole ("HOBT") active esters. Arginine was coupled via a DCC-mediated procedure. The Kaiser test (Kaiser, *Anal. Biochem.*, 34:595, (1970)) was used to monitor the efficiency of individual couplings with double-couplings performed, if necessary. Following deprotection of the final amino acid of the sequence, the N-terminal amino groups were actylated by the addition of a solution of 10% acetic anhydride, 5% diisopropylethylamine in CH_2C1_2. Peptides were cleaved from resin with either HF/Anisole/ Ethanedithiol or the 'low-high' procedure of Tam, et al., *J. Am. Chem. Soc.*, 21:6442, (1983).

Purification of Peptides

Following HF cleavage, the resin-peptide was washed with ethyl ether and the peptide extracted with 10% acetic acid. The resultant crude peptide was chromatographed on a Sephadex G-25 gel filtration column with 10% aqueous acetic acid as eluant. Peptides were detected spectrophotometrically at 280 nm. Fractions from the main UV absorbing band were pooled and lyophilized. Subsequently, the peptides were purified by preparative HPLC using a Vydac C-18 column (21 X 250 mm). Peptides were eluted from the column with a 0.05% trifluoroacetic acid buffer and a 25-45% acetonitrile gradient over 40 minutes. The flow rate was 10 ml/min, and a typical load was 50-100 mg. Fractions were isolated from the peptide eluant by U.V. detection at 235 nm. The resultant fractions were lyophilized and analyzed for purity by analytical HPLC (Beckman System Gold) with a 100 mm $NaC10_4$ and 0.1% $H3PO_4$ pH 2.5 buffer and a acetonitrile gradient of 30-50% over 30 minutes. Peptides achieved by this process were > 98% in purity.

Characterization of Peptides

Acid hydrolysates were prepared by treating peptides with 6N HCl *in vacuo* at 110°C for 24 hours. Cysteine content was determined after conversion to S-carboxymethyl cysteine, tryptophan content after hydrolysis with 4N methanesulphonic acid. Hydrolyzed samples were analyzed on a Beckman 6300 amino acid analyzer interfaced with a Nelson 3000k data system. Norleucine was used as an internal standard.

EXAMPLE 2: COUPLING OF PEPTIDE TO CARRIER

Coupling Via Glutaraldehyde to Carrier for Immunization

Peptides B138, 1005/45 and 1047/51M were coupled to both keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) by dissolving the carrier in PBS to a final concentration of 1 mg/ml. Insoluble aggregates of carrier were removed by filtration through a 5µm syringe filter. Concentration of the filtrate was determined by absorbance at 280 nm, and peptide was added by weight to yield a 100X molar excess of peptide to carrier. Glutaraldehyde (1%) in solution was added to the stirred volume of peptide/carrier to a final concentration of 0.1% glutaraldehyde. The reaction solution was allowed to stir at room temperature until a cloudy ppt began to form (~1.5 hrs.) at which time the reaction was quenched by the addition of 500 µl of 2M glycine per 10 ml of solution. The conjugate was immediately dialyzed versus PBS for 24 hours. The conjugate was removed from dialysis, sterile filtered (0.2µm) and stored at 2-8°C.

Coupling to Carrier Via Heterobifunctional Reagent

Peptides 1005/45 and B119 were conjugated to KLH and BSA utilizing amino groups on the carrier and the terminal cysteines on the peptide. The carrier in solution, at a concentration of 1 µg/ml, was treated with a 200 fold molar excess of sulfosuccinimidyl 4-(N-maleinidomethyl) cyclohexane-1-carboxylate ("Sulfo-SMCC," a hetrobifunctional reagent available from Pierce Biochemicals, Rockford, IL) in PBS, for two hours at room temperature with stirring (Hashida, S., *et al.*, *J. Applied Biochem.*, 6:56 (1984)). The derivatized conjugates were dialyzed overnight against PBS to remove free heterobifunctional reagents. Peptide was reduced by the addition of 50 mM dithiothreitol ("DTT")

and incubation at 37°C for 1 hour. The reduced peptide was separated from reductant by a Sep-Pak (Waters) apparatus and subsequently eluted with 95% CH_3CN directly into dialyzed, derivatized carrier. The reaction was achieved under argon blanket and argon saturated solutions for two hours at room temperature with stirring. Free peptide was removed from the conjugate by dialysis.

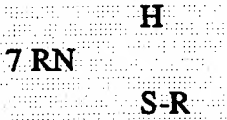
Reaction Scheme

NHS Ester: (Carrier)

R-C-O-N + RNH_2 PH>7 R-C-N-R+HO-N

Cysteine Peptides

Maleimide Rx Scheme (peptides)



Additional Coupling Schemes

Peptides lacking a terminal Cys are coupled to carriers by the derivatization of the carrier to introduce a sulfur atom with either 2-iminothiolane or S-acetylmercaptosuccinic anhydride. An N-hydroxysuccinimide-maleimide linker can be utilized targeting the primary amines of the peptide, and the sulfo-derivatized carrier. Additional methods include carbodiimide-mediated coupling through the carboxyl group of the peptide and the primary amines of the carrier (Staros, *et al.*, *Anal. Biochem*, 156:220 (1986)). Tetanus toxoid was conjugated to peptide utilizing the aforementioned methods.

EXAMPLE 3. CYCLIZATION OF PEPTIDE 1005/45

Cyclization of the 1005/45 peptide through its terminal cysteines may enhance its conformational rigidity. Therefore, after initial reverse phase HPLC purification as previously described, peptide 1005/45 was cyclized by the formation of a disulfide bond between the terminal cysteines. Cyclization was effected by first reducing the SH groups in the peptide with 50 mm DTT. The peptide was separated from reductant by elution from a Sep-Pak apparatus with 95% CH_3CN. In this manner, 20 mg of reduced 1004/45 was directly introduced to 500 ml of

PBS, pH 7.5. A solution of 400 mg of $K_3Fe(CN)_6$ in 200ml distilled H_2O was added dropwise to the stirred solution until the yellow color was maintained. The solution was allowed to stir vigorously overnight at room temperature. The entire reaction solution was then pumped onto a preparative 21 X 250 mm reverse phase C-18 HPLC column and eluted with acetonitrile. The resultant cyclized peptide was analyzed by HPLC for purity as described previously. Confirmation of the cyclic monomer was determined by size exclusion chromatography on a Hewlett-Packard PLgel 5µm 100 Angstrom size exclusion column. The monomeric cyclic peptide eluted as a single peak of approximately 3,000 M.W. Alternative synthesis methods for cyclic 1005/45 includes the introduction of acetomidomethyl groups to to the cysteine residues. Cyclization is achieved by the removal of the ACM group with NaI in solution (Stewart, J.M., *et al.*, *Solid Phase Peptide Synthesis* 2nd Edition:95-96, Pierce Chemical Co.). To enhance the stability of the peptide under physiological conditions, the disulfide bond may be replaced with either a peptide bond or a homobifunctional linker, *e.g.*, bis maleimide.

EXAMPLE 4: BIOLOGICAL PROPERTIES OF GP120 RELATED PEPTIDES

Bioassays: Infectivity and Syncytial Blocking

Owing to typically low affinities of peptides for antibodies and receptors, bioassays were utilized to evaluate peptides derived from this region of gp120 as potential inhibitors of CD4 dependent cellular functions. The 18mer tract extending from residue 421 to 438 and several other synthetic peptides from the 413-456 region were first assayed for their ability to inhibit HIV-1 and HIV-2 infection of $CD4^+$ cells. Both $CD4^+$ T cells (VB) and B cells (AA5) were used as target cells in the infectivity assay. As shown in FIG. 2, the BRU (B138) isolate 421-438 residue sequence was the most effective of the peptides assayed to block infectivity of the $CD4^+$ cell lines by the HIV-1 isolate $_{IIIB}$, LAV-1, SF33, and HIV-2. Cells were greater than 95% viable as tested by Trypan Blue exclusion and ^{51}Cr release assays. The 421-438 peptide (1005-34) derived from the $_{IIIB}$ isolate was less effective in inhibiting infection as determined by syncytial formation utilizing the HXB2 cell line. These two peptides (B138 and 1005-34) differ at positions 423

and 429. An analogous SIV HIV-2 consensus 18 residue peptide (1017-33) from the SK6W isolate did not block infectivity of the cell lines by the $_{IIIB}$ isolate. To what extent the substitutions at positions 421, 426, 428 and 433-434 in the consensus peptide effects the CD4 binding conformation available to this peptide remains to be examined. As part of applicants' conformational studies of the CD4 binding region, a synthetic peptide (B92) with four residue substitutions at the highly conserved positions 426, 428, and 430-431 of HIV-1 isolates within the 18 residue tract of B138 was shown to lack the syncytial blocking activity of B138. These positions were chosen for the introduction of residue substitutions based upon sequence relationships identified by the applicants between the purported CD4 binding domain of gp120 and the immunoglobulin supergene family. Sequence identities between the 413-456 gp120 region and kappa chain sequence of immunoglobulins is of particular interest since it has been shown that a kappa light chain dimer from a parental monoclonal G17-2 can bind to CD4.

As shown in FIG. 1, in addition to a control peptide, a major histocompatibility complex (MHC) class II-derived peptide from the beta 1 domain (1005/03) containing the suggested adhesiotope sequence RFDS was also assayed for syncytial blocking and failed to block syncytia. This peptide has exhibited immunosuppressive activity of lymphocytes stimulated by influenza hemagglutinin. It has been proposed by others that the human lymphocyte antigen (HLA) class II and CD4 may interact through the charged residues of this tetrapeptide type since an analog of the sequence RADS is present in the NH2 terminal immunoglobulin V-like domain of CD4. The RADS tract in CD4 is adjacent to the purported binding site on CD4 for gp120, however, as shown in FIGURE 1 the CD4 derived peptide (1005/78) that contains this tract also did not inhibit syncytia. Indeed, reports by others have determined that the gp120 contact sites on CD4 are distinct from those which bind MHC class II molecules.

Syncytial blocking activity has been equated with the blocking of gp120/CD4 binding (see, Lifson, J.D., *et al.*, *Nature*, 323:725 (1986)) As shown in FIG. 2, flow cytometry analysis indicated that B138 can inhibit the binding of the two virus isolates examined at a concentration equal to that for syncytial blocking. B138 peptide at 100 µg/ml appears to

inhibit binding of both $_{IIIB}$ and $_{RF}$ isolates to CEM cells. The control peptide 1000-05 did not inhibit the fluorescence staining.

Lymphocyte Responses to B138

Generally, T lymphocyte responses to HIV antigens, characterized by lymphocyte proliferation or interleukin release are only detectable to a modest degree *in vitro* in individuals or chimpanzees infected with the AIDS retrovirus. To a large extent these findings are probably dependent on the clinical time course of disease progression. Applicants examined the proliferation of lymphocytes (peripheral blood mononuclear cells; PBMCs) in response to peptide B138 in the presence or absence of rIL-2 in HIV-seronegative and HIV-seropositive individuals. As shown in FIG. 3, only very low levels of proliferation of PBMCs from four HIV-seronegative individuals were observed in response to any concentration of B138 (mean SI = 1.5 $\pm$0.2) and these responses were not significantly augmented by rIL-2. Proliferation of lymphocytes from HIV-seropositive asymptomatic individuals and AIDS-related complex (ARC) patients in response to B138 (0.01-100 µg/ml) was also less than three-fold above background, even in the presence of rIL-2.

Immunosuppression by B138

As an extension of previous studies by others demonstrating the immunosuppressive properties of gp120, applicants also analyzed the ability of the 421-438 (B138) peptide to inhibit antigen-derived proliferation of human lymphocytes. Preliminary experiments involving PBMCs from HIV-seronegative asymptomatic donors cultured with tetanus toxoid and/or B138, indicate that at concentrations of 100 µg/ml, B138 inhibited lymphocyte proliferation in response to tetanus toxoid by an average of 65%. To further characterize the suppressive effect of B138 on lymphocyte proliferation, applicants examined the dose response of B138 in suppressing tetanus toxoid, CMV, and PHA lymphocyte proliferation of PBMCs from HIV-seronegative donors. As shown in Table 4 below, B138 inhibited both tetanus toxoid and CMV in a dose-dependent manner.

TABLE 4

SUPPRESSION OF ANTIGEN-DRIVEN LYMPHOCYTE PROLIFERATION BY B138

Peptide concentration (μg/ml)	Tetanus Toxoid	CMV
1.0	94%	92%
12.5	70%*	109%
25.0	66%*	75%*
50.0	59%*	67%*
75.0	N.D.	46%*
100.0	53%*	46%*

Percentage of maximal stimulation of normal lymphocytes cultured with antigen and peptide B138

Maximal stimulation with tetanus toxoid (2.5 Lf/ml) in the absence of peptide: 19,303 cpm

Maximal stimulation with CMV (10μg/ml) in the absence of peptide: 30,609 cpm

* $p < 0.01$ (Student's t test) in comparison with cultures stimulated with antigen alone.

However, neither B138, nor control peptides suppressed PHA stimulation over the concentration range examined. The suppression of tetanus toxoid and CMV was not affected by the addition of rIL-2. These studies demonstrated that at high concentrations peptide B138 could inhibit proliferation of lymphocytes from normal donors in response to a recall antigen.

The immunosuppressive properties of B138 parallel those observed by others in the suppression of immune responses by glycosylated gp120,

except B138 does not inhibit PHA lymphocyte proliferation. In contrast, non-glycosylated, yeast-expressed, gp120 has been shown by others not to inhibit PHA or antigen-driven lymphocyte proliferation. Infection by viruses such as CMV or influenza characteristically induces lymphocytes capable of proliferating *in vitro* to the sensitizing antigens. These proliferative responses are generally associated with the activity of $CD4^+$ helper T lymphocytes. Applicants' studies and others indicate that gp120 binding to CD4 interferes with these proliferative responses. This conclusion also parallels observations that lymphocytes from nearly all AIDS patients as well as from some HIV-infected individuals are unable to proliferate in response to recall antigens and these responses cannot generally be reconstituted with rIL-2. The suppressive effects of B138 on mitogen-driven proliferation could not be augmented by rIL-2 even in HIV-seronegative donors. It has been suggested by others that such observations are the result of selective defects in cell-mediated immune responses to HIV antigen. Therefore, applicants postulate that the 421-438 residue sequence from the cell attachment site of gp120 is responsible for some of the *in vitro* characteristics of native gp120. However, it is not clear whether B138 *in vitro* effects are the result of binding to and modulating the CD4 receptor. Although applicants do not wish to be bound by any theory, it may be that B138 modulates the cellular metabolism of the target cells in an undefined way which deactivates or reduces cell proliferation, or the target cells susceptibility to cell-to-cell fusion by syncytia.

Anti-HIV Response of B138

The immunogenicity of B138 was determined by subcutaneous inoculation of mice and rabbits. As shown in FIG. 4, mouse anti-sera to B138 was shown to preferentially bind to $IIIB$-infected H9 cells as assessed by FACS analysis. This rabbit sera could immunoprecipitate purified gp120.

Applicants assessed the reactivity of B138 with sera (1:4 dilution) from HIV seronegative and HIV seropositive, asymptomatic study subjects. As shown in FIG. 5, only very low levels (background) of reactivity to B138 were detected using sera from 19 HIV seronegative individuals. Only 2 of 18 (11%) HIV seropositive, asymptomatic individuals had levels of anti-B138 antibodies that were significantly different

($p<0.003$) from HIV$^-$ controls. Thirty-three percent of ARC patients (7 of 21) and 10% (2 of 20) of AIDS patients had significant levels of antibodies that bound B138. Only one HIV-infected individual, an ARC patient, had levels of anti-B138 antibodies that were significantly different from HIV$^-$ asymptomatic individuals at 1:32 dilutions of serum ($p<0.003$). These results demonstrate that HIV-1 does not stimulate the production of antibodies that bind B138 in most infected individuals. Furthermore, in the few individuals that had detectable levels of anti-B138 antibodies, these antibodies occurred at relatively low titers.

In rabbits immunized with both the B138 and 1005/45 synthetic peptides, neutralizing antibodies were detected after the second and third immunizations (see Table 5). Rabbits were inoculated with 100 mg of peptide in SAF adjuvant on each occasion. Serum was separated from clotted blood and heat inactivated (56°C for 30 minutes). The ability to neutralize virus infection was determined by standard procedures using the AA% target cell line and the $HTLV_{IIIB}$ isolate of HIV. Infection was assessed by measuring p24 *gag* core antigen in the cell cultures after seven days (Whalley A.S., *et al.*, *Vaccines* (1989), Cold Spring Harbor Press).

TABLE 5

NEUTRALIZATION OF HIV BY POLYCLONAL ANTISERA GENERATED BY gp120 SYNTHETIC PEPTIDES

	% Neutralization*	
Sample	*2d7***	*3d7****
B138-1	22	89
B138-2	22	86
1005/45-1	28	65
1005/45-2	0	93
343-1	0	0
343-2	21	0

* % neutralization = 1-(pg/ml p24 *gag* antigen with test sera/ (pg/ml p24 *gag* antigen with prebleed sera)
** measurements taken seven days after second immunization.
*** measurements taken seven days after third immunization.
Assays carried out using sera at a dilution of 1:7. Anti-343 sera generated against a recombinant fragment of gp120 (spanning amino acids 343 to 511).

EXAMPLE 5: SOLID SUPPORT PREPARATION FOR GENERATION OF ANTIBODIES

Reacti-Gel Support

Following the manufacturer's recommendations, we have utilized Reacti-Gel™ to prepare an affinity column linked with the B138 peptide to purify antibodies from mice immunized with B138. Specifically, the peptide was coupled to Reacti-Gel™ by the following procedure: 5.1 mg B138 was dissolved in 7ml 0.1M borate buffer, pH8.5. 10 ml of Reacti-Gel™ 6X was dried under vacuum on a sintered glass funnel and added

to the peptide solution; the peptide containing the mixture was then rotated for 48 hours at 4°C. After this time the presence of amine groups on the reacted gel was determined with ninhydrin reagent.

Affi-gel 401 Coupling of Peptide With Bis-Maleimide Linker

Additionally, peptide 1005/45 was coupled to Affi-gel 401 affinity resin (BioRad). The functional SH group on the support is designed to form a reducible disulfide bond with SH groups on the ligand. We coupled peptide 1005/45 to Affi-gel 401 utilizing the intrinsic terminal cysteines along with the incorporation of the pH stable, nonreducible linker bis-maleimidomethy ether. Our intent was to replace the disulfide bond with a "spacer" to reduce steric inhibition of antibody binding, along with minimal restriction of the peptide in solution, enabling the peptide to adopt a favorable tertiary conformation. In this way, the incorporation of the bis-maleimide linker may facilitate the coupling of the ligand/support and allows dissociation of the anti-peptide antibody by pH adjustment, the support can then be be re-used. Incorporation of linker with support and peptide is summarized as follows. Affi-gel 401, containing 5.1 µM of functional groups per ml of gel was washed extensively in a sintered glass funnel. A 100 x molar excess of bis-maleimidomethy ether per mole of functional groups on the Affi-gel support was added to the Affi-gel solution in 200 µl CH_2Cl_2. The reaction vessel was sealed under argon and allowed to react for two hours at room temperature. The reaction mixture was transferred to a sintered glass funnel and again washed with CH_2Cl_2 extensively.

The derivatized Affi-gel in 5 ml of CH_2Cl_2 was transferred to a reaction vessel to which was added a 10x molar excess of reduced peptide to functional groups on the resin. The reaction solution was sealed under argon and allowed to react overnight at room temperature with stirring. Residual unreacted -SH groups were capped by the addition of 100x molar excess of N-ethyl-maleimide. Capping was allowed to proceed for 1 hour at room temperature with stirring. The resin/peptide was washed with PBS pH 7.2 and stored at 2-8°C.

Additional Supports

Other techniques include carbodiimide-mediated coupling targeting the carboxyl or amino termini. Additionally, in-house derivatizations of

commercially available supports, along with the incorporation of the appropriate homobifunctional or heterobifunctional linker may be attempted.

EXAMPLE 6: GENERATION OF GP120 SURROGATE ANTIBODIES USING PEPTIDE COLUMN

One of the embodiments of the present invention is the use B138 or a related synthetic peptide coupled to a solid support as a means to isolate antibodies that may be used to generate anti-idiotypic antibodies that may ultimately be used to elicit active anti-HIV immune responses in individuals infected with the AIDS virus or even as a prophylactic vaccine. The procedure for carrying out this method follows.

Source of Anti-Peptide Antibodies

Antibodies are isolated from the sera or plasma of HIV-infected primates (including humans or chimpanzees) or small vertebrate animals such as rodents or rabbits which have been immunized with either the peptide described herein or the gp120 molecule or fragments thereof.

Isolation of Anti-Peptide Antibodies

An affinity column comprising the HIV envelope-derived peptide coupled to a solid phase matrix or support is prepared according to the procedure described in Example 5. The antibodies are isolated from the serum or plasma of HIV-infected primates (including humans or chimpanzees) or small vertebrate animals such as rodents or rabbits that have been immunized with either the peptides described herein or the gp120 molecule or fragments thereof. In a first isolation step, an immunoglobulin fraction of the serum or plasma is precipitated in the presence of 50% saturated ammonium sulfate (Hudson L. and Hay, F.C., *Practical Immunology*, 3rd edition, Blackwell Scientific Publications, Oxford, 1989). The precipitated fraction is washed with ammonium sulfate and redissolved in a convenient volume of a physiologically neutral buffer, such as phosphate buffered saline (PBS), pH7.2, and passed over the column. Continuous recycling of the immunoglobulin fraction over the column for an extended period of time (18 hours or overnight) is preferred in order to achieve maximum levels of antibody

binding to the peptide. Such a procedure is facilitated by the use of a peristaltic pump. The column is then washed with several volumes of PBS in order to remove passively adsorbed, non-peptide specific antibodies, and serum proteins. The anti-peptide antibodies bound to the column are eluted at low pH, for example by washing the column with 0.1M glycine-HCl buffer, pH 2.5 to 4.0. All column eluates (including washes) are passed through an ultraviolet detection unit in order to monitor their relative concentrations of protein. Alternative eluting agents include solutions of high salt concentration, *e.g.*, 2M sodium chloride or chaotropic agents such as 6M guanadine hydrochloride. Finally, the pH of the antibody preparation (referred to as the first plurality of antibodies or Ab1) is neutralized with a basic reagent such as Tris.

Preparation and Selection of Anti-Idiotypic Antibodies

The following procedure is used to prepare anti-idiotypic antibodies (also termed the second plurality of antibodies or Ab2), which are candidates for immunotherapy or vaccines. After first determining the fidelity of the purified antibodies in peptide ELISA and gp120 ELISA, they are used to immunize small animals.

Preparation of Monoclonal Anti-Idiotypic Antibodies

At least two options are available here. The preferred technique is to immunize mice with the antibody contained in an immunogenic carrier such as Freund's adjuvant according to the standard protocol. After 4-6 weeks, the mice are bled and the serum anti-idiotype titre determined by ELISA. If the concentrations of these antibodies is substantial, the mice are sacrificed, their spleens removed and fused with a suitable cell line, such as SP/2 (Shulman, M., Wilde, C.D., Köhler, G., *Nature*, 276: 271-72 (1978)), and monoclonal antibodies prepared and selected according to the standard procedures previously described. Following this procedure, a sub-population of anti-idiotypic antibodies are screened for those bearing the "internal image" of the antigen to which an immune response is desired, i.e., these anti-idiotypes are the antigen surrogates or mimics and will be the candidates for immunotherapeutics and vaccines. Selection of candidate internal image-bearing antibodies (also called Ab2 betas) is performed by assessing the ability of either immunizing peptide or gp120 or both of

these molecules to inhibit the binding of Ab2 to Ab1 in an ELISA. Candidate Ab2 beta antibodies are characterized by their ability to induce anti-peptide and anti-HIV responses (including anti-gp120 neutralizing antibodies in another animal species, preferably rabbits).

Generation of Polyclonal Anti-Idiotypic Antisera

An alternative to generation of monoclonal Ab2s is to produce polyclonal anti-idiotypic antibodies. As such, the purified Ab1s described above are inoculated in an immunogenic formulation into rabbits according to the method of Kennedy and Dreesman (Kennedy R.C., *et al., J. Virol. Meth.*, 7:103-15 (1983)).

Preparation of Antisera

The animals are hyperimmunized with selected antibodies as described above. Initially they are injected intramuscularly with Ab1 emulsified in Freund's complete adjuvant. This is followed by a second injection, approximately one week later, with the antigen in Freund's incomplete adjuvant. Subsequent booster inoculations are made with the antibody precipitated on alum to increase its immunogenicity and to improve the chances of obtaining internal image anti-idiotypes. As many as eight injections of idiotype (approximately 100 µg per inoculation, given at 7-10 day intervals may be necessary to produce anti-idiotypes) (see Kennedy and Dreesman, *supra*). After 6-8 weeks and appropriate test bleeds to determine the anti-idiotype titer, the rabbits are exsanguinated. Previous studies which have used rabbits to raise anti-idiotype antisera have indicated that the majority (approximately 80%) of the antibodies produced are anti-isotypic, about 15% are anti-allotypic, and probably no more than 5% are specific for the idiotype (see, Kennedy and Dreesman). Thus, anti-idiotype antibodies are isolated by extensive absorption on affinity columns (Sepharose 4B, covalently-linked with human or immunoglobulins, depending on the origin of the Ab1) to remove non-idiotypic specificities. The absorptions on 10 ml columns of Reactigel™ (Pierce Biochemicals, Rockford, IL) that is coupled with either human or mouse immunoglobulin depending on the source of Ab1. Ammonium sulfate preparations of the whole anti-idiotype antisera are applied to the columns and recirculated by means of a peristaltic pump (5-10 ml per hour) for 18 hours at 4°C. After suitable washing of the bound, non-

idiotype associated antibodies with glycine-HCl buffer, pH 2.5, and PBS, the column may be used for further absorptions.

Screening and Characterizing Anti-Idiotype Antibodies

A fundamental premise of applicants' approach is that anti-idiotype antibodies will be recognized as antigen-inhibitable by the immunizing idiotype. Thus, it is preferred to screen for and determine anti-idiotype activity using the following solid-phase inhibition assay that has been used to study other idiotype-anti-idiotype and viral antigen interactions.

Ninety-six-well microtiter plates are coated with the idiotype antibody used for the immunization procedure. The inhibition of binding of ^{125}I-labeled gp120 by the simultaneous addition of the anti-idiotype antibodies is measured. However, it is recognized that the antigen inhibition assay described above does not discriminate between antibodies with specificity for idiotypic determinants very close to, if not identical with, the paratopic regions of Ab1 (the Ab2 gamma subset) and true internal image anti-idiotypes (Ab2 beta antibodies). Thus, the ability of an Ab2 antibody to inhibit antigen binding is a necessary, but insufficient condition to identify it as an "internal image" anti-idiotype. Therefore, in order to determine more precisely whether the anti-idiotypes generated according to the present invention recognize true internal images of a HIV epitopes, their ability to recognize cross-reactive, interspecies idiotypes is examined. This characteristic is examined using the technique of Kennedy, R.C., *et al.*, *Eur. J. Immunol.*, 13: 232-5 (1983)). In a solid-phase radioimmunoassay, the anti-idiotype (Ab2) is coated onto wells of a microtiter plate and after the conventional washing and blocking steps, ^{125}I-labeled idiotype is added to the wells. After incubation (one hour at room temperature) and washing, the plates are washed and the bound radioactivity counted in a gamma ray spectrometer. The source of the idiotype is rabbits and mice hyperimmunized with HIV according to conventional techniques, as well as sera from HIV-infected chimpanzees and humans. They are radiolabeled with ^{125}I using the iodogen method. Should an Ab2 bind to anti-HIV idiotypes from more than one species there is a strong likelihood that the anti-idiotype bears the internal image of the antigen.

Rats are the preferred species for evaluating candidate Ab2 beta antibodies generated by the polyclonal technique.

Testing the Antigenicity of Anti-Idiotype Antibodies *in vivo*

Once Ab2 beta anti-idiotypes are obtained by the *in vitro* testing, their ability to elicit anti-HIV humoral and cellular responses *in vivo* is examined. Rats or rabbits are used for these experiments as their size allows us to obtain sufficient sera to test for anti-HIV antibodies by immunoblotting and neutralizing antibodies. The experimental protocol is as follows:

Immunizations

At least 20 animals are needed to test any particular anti-idiotype preparation. Ten are on test and inoculated with anti-idiotype and ten serve as controls and are inoculated with the same quantity of immunoglobulin from the same species as the anti-idiotype. The inocula comprises at least 50μg of immunoglobulin emulsified in adjuvants or adsorbed onto alum as discussed above. Between 3-6 injections of anti-idiotype, given at weekly intervals, are necessary to immunize the animals. Test bleeds are made four weeks after the initial inoculation and at weekly intervals thereafter.

Humoral Responses

The anti-idiotype inoculated animals are screened for the presence of anti-HIV antibodies by the gp120 ELISA and the specificity of the response is measured by immunoblotting or radioimmunoprecipitation. Neutralizing activity (as described previously) is determined against multiple HIV isolates.

EXAMPLE 7: GENERATION OF GP120 SURROGATE ANTIBODIES USING PEPTIDE AS AN IMMUNOGEN

An additional embodiment of the present invention is the use 1005/45 or B138 as an immunogen to elicit antibodies in an immunized animal species that may be purified on gp120 affinity columns and thus provide a source of Ab1s, which in turn be used to generate Ab2 anti-idiotype antibodies, which are candidates for vaccines or immunotherapeutics for HIV infection.

Immunizations

Small animals, preferably rabbits, are used for the primary immunizations. It is also possible that goats may be utilized as they are capable of providing relatively large quantities of sera; an important practical consideration. The peptide may be tried in several formulations of carrier and adjuvant in order to improve its immunogenicity. In preferred formulations, various combinations of carrier (specific- and glutaraldehyde cross-linked KLH) and adjuvant (Freund's complete and incomplete, as well as muramyl dipeptides, such as SAF) are utilized.

Isolation of Anti-gp120 Antibodies

An affinity column comprising the gp120 portion of the HIV envelope is coupled to a solid phase matrix according to the method described in Example 5. Antibodies are isolated from the serum or plasma of small vertebrate animals such as rodents or rabbits which have been immunized with synthetic peptides of the present invention. The anti-gp120 antibodies are isolated in essentially the same manner described in Example 6.

Generation of Ab2

Once the sub-population of anti-peptide antibodies that are specific for gp120 has been isolated, both monoclonal or polyclonal anti-idiotypic antibodies are prepared by the methods described in Example 6.

Testing of Ab2

Both the *in vitro* and *in vivo* evaluation of the Ab2 is performed as described in Example 6.

We Claim:

1. A compound characterized by the capability of eliciting and/or binding with HIV neutralizing antibodies, wherein said capability results from an amino acid sequence of at least 17 amino acid residues which:

(a) is a neutralizing epitope(s) from the CD4 binding region of the gp120 envelope protein of an HIV isolate;

(b) is a portion or segment of said epitope; or

(c) is an immunological equivalent of said epitope or portion thereof.

2. A compound, other than a naturally occurring HIV envelope protein, characterized by the capability of eliciting and/or binding with HIV neutralizing antibodies, said compound comprising the neutralizing epitope(s), or segment thereof, from the CD4 binding region of the gp120 envelope protein of an HIV isolate; wherein said neutralizing epitope comprises at least 17 amino acid residues.

3. A compound according to claim 1 or 2, wherein said CD4 binding region is at or about amino acid position 335 through at or about amino acid position 517.

4. A compound according to claim 1 or 2, wherein said isolate is the MN isolate.

5. A compound according to claim 1 or 2, wherein said HIV neutralizing antibodies are group-specific.

6. A compound characterized by the capability of eliciting and/or binding with HIV neutralizing antibodies, said compound comprising a peptide of at least 17 to about 100 amino acid residues, said peptide selected from the group consisting of:

(a) a peptide comprising a segment or the entirety of an amino acid sequence selected from the group:

(1) B138:
KQFINMWQEVGKAMYAPP;

(2) 1029/16:
KQFINMWQEVGKAMYAPPISGQIR;

(3) 1005/45:
CRIKQFINMWQEVGKAMYAPPISGQIRC;

(4) 1029/16:
KQFINMWQEVGKAMYAPPISGQIR;

(5) 1029/76:
KQFINIWLEVGKAMYAPP;

(6) 1029/77:
KQFINTWLHEVGKAMYAPP;

(7) 1029/81:
KQFINMWLEVGKAMYAPP;

(8) 1029/82:
KQFINIWQEVGKAMYAPP;

(9) 1029/83:
KQFINMWREVGKAMYAPP;

(10) 1029/84:
KQFINTWQEVGKAMYAPP;

(11) 1029/85:
KQFINMWQEVGKNVYAPP;

(12) 1029/86:
KQFINMWQEVGKAVYAPP;

(13) 1005/28:
KQFINMWQE;

(14) 1005/33:
GDSTITLPCRIKQFINMWQE;

(15) 1017/08:
GDSTITLPCRIKQFINMWQEVGKAMYAPP;

(16) 1017/88:
PPISGQIRCSSNITGLLLTRDGG;

(17) 1005/34:
KQIINMWQKVGKAMYAPP;

(18) 1005/70:
GDSTITLPCRIKQIINMWQK;

(19) 1005/29:
KQIINMQKVGKAMYAPP;

(20) 1139/43:
KQIINMWQEVGKAMYAPP;

(21) 1029/04:
RQIINTWHKVGKNVYLPP;

(22) 1043/68:
CRIKEIINMWQEVGKAMYAPPIKGQVKC;

(23) 1043/72:
CRIKQIINRWQVVGKAMYALPIKGLIRC;

(24) 1077/36:
CHIRQIINTWHKVGKNVYLPPREGDLTC;

(25) 1103/01:
KQIINMWQEVGKAMYAPPIEGQIR;

(26) 1103/02:
QMHEDIISLWDQSLKKQIINMWQEVGKAMYAPPIEGQIR;

(27) 1103/68:
CGGEFFYGGKIKQIINMWQEVGKAMYAPPIEGQIRC;

(28) 1139/35:
SSNITGLLLTRDGGKDTDTNDTEIFRPGGGDMRDNWRS;

(29) 1139/60:
NKTIVFNQSSGGDPEIVMHSFNCGGEFFYGGKIKQIINMWQEVG-KAMYAPPIEGQIRCSSNITGLLLTRDGGKDTDTNDTEIFRPGGGD-MRDNWRS;

(30) 1139/64:
KQIINMWQEVGKACYAPPIEGQIRC

(31) 1139/65:
KQIINMWQEVGKACYAPPIEGQIRCSSNITGLLLTRDGG

(32) 1139/63:
FYCNTSPQLFNSTWNGNNTWNNTTGSNNNITLQCKIKQIINMWQ-EVGKAMYAPPIEGQIRCSSNITGLLLTRDGG; and

chemical modification thereof; and

(b) an peptide analog comprising said sequence, wherein amino acid residues are modified, substituted, deleted, inserted, or added to said sequence, provided that the immunoreactivity of said analog to anti-peptide antibodies, wherein said antibodies also are reactive with gp120, is preserved in said analog.

7. A peptide selected from the group consisting of:

(1) B138:
KQFINMWQEVGKAMYAPP;

(2) 1029/16:
KQFINMWQEVGKAMYAPPISGQIR;

(3) 1005/45:
CRIKQFINMWQEVGKAMYAPPISGQIRC;

(4) 1029/16:
KQFINMWQEVGKAMYAPPISGQIR;

(5) 1029/76:
KQFINIWLEVGKAMYAPP;

(6) 1029/77:
KQFINTWLHEVGKAMYAPP;

(7) 1029/81:
KQFINMWLEVGKAMYAPP;

(8) 1029/82:
KQFINIWQEVGKAMYAPP;

(9) 1029/83:
KQFINMWREVGKAMYAPP;

(10) 1029/84:
KQFINTWQEVGKAMYAPP;

(11) 1029/85:
KQFINMWQEVGKNVYAPP;

(12) 1029/86:
KQFINMWQEVGKAVYAPP;

(13) 1005/28:
KQFINMWQE;

(14) 1005/33:
GDSTITLPCRIKQFINMWQE;

(15) 1017/08:
GDSTITLPCRIKQFINMWQEVGKAMYAPP;

(16) 1017/88:
PPISGQIRCSSNITGLLLTRDGG;

(17) 1005/34:
KQIINMWQKVGKAMYAPP;

(18) 1005/70:
GDSTITLPCRIKQIINMWQK;

(19) 1005/29:
KQIINMQKVGKAMYAPP;

(20) 1139/43:
KQIINMWQEVGKAMYAPP;

(21) 1029/04:
RQIINTWHKVGKNVYLPP;

(22) 1043/68:
CRIKEIINMWQEVGKAMYAPPIKGQVKC;

(23) 1043/72:
CRIKQIINRWQVVGKAMYALPIKGLIRC;

(24) 1077/36:
CHIRQIINTWHKVGKNVYLPPREGDLTC;

(25) 1103/01:
KQIINMWQEVGKAMYAPPIEGQIR;

(26) 1103/02:
QMHEDIISLWDQSLKKQIINMWQEVGKAMYAPPIEGQIR;

(27) 1103/68:
CGGEFFYGGKIKQIINMWQEVGKAMYAPPIEGQIRC;

(28) 1139/35:
SSNITGLLLTRDGGKDTDTNDTEIFRPGGGDMRDNWRS;

(29) 1139/60:
NKTIVFNQSSGGDPEIVMHSFNCGGEFFYGGKIKQIINMWQEVG-KAMYAPPIEGQIRCSSNITGLLLTRDGGKDTDTNDTEIFRPGGGD-MRDNWRS;

(30) 1139/64:
KQIINMWQEVGKACYAPPIEGQIRC

(31) 1139/65:
KQIINMWQEVGKACYAPPIEGQIRCSSNITGLLLTRDGG

(32) 1139/63:
FYCNTSPQLFNSTWNGNNTWNNTTGSNNNITLQCKIKQIINMWQ-EVGKAMYAPPIEGQIRCSSNITGLLLTRDGG; and

chemical modification thereof.

8. An immunoglobulin composition comprising an immunoglobulin or fragment thereof and a compound according to claim 1 or 2, wherein said compound is incorporated in said immunoglobulin or said fragment.

9. A method for generating polyclonal antibodies for use as vaccines or in immunotherapy, comprising the steps of:

(a) coating a solid support with an effective amount of composition comprising a compound according to claim 1 or 2;

(b) applying a serum sample containing anti-gp120 or anti-compound antibodies, wherein a first plurality of said antibodies in said sample complex with said compound, separating said solid support from said serum sample, and eluting said first plurality of antibodies complexed to said compound from said solid support;

(c) immunizing a first mammalian host with an immunogenic formulation comprising said first plurality of antibodies; and

(d) selecting and purifying a second plurality of polyclonal antibodies from the sera of said first host, wherein said second plurality of polyclonal antibodies are characterized by their immunological reactivity with said first antibodies and immunological competition with gp120.

10. A method for generating a monoclonal antibody for use as vaccines or in immunotherapy, comprising the steps of:

(a) coating a solid support with an effective amount of composition comprising a compound according to claim 1 or 2;

(b) applying a serum sample containing anti-gp120 or anti-compound antibodies, wherein a first plurality of said antibodies in said sample complex with said compound, separating said solid support from said serum sample, and eluting said first plurality of antibodies complexed to said compound from said solid support;

(c) immunizing a first mammalian host with an immunogenic formulation comprising said first plurality of antibodies;

(d) generating a hybridoma from an antibody producing organ of said first host, wherein said hybridoma is capable of producing a monoclonal antibody characterized by its immunological reactivity with said first plurality of antibodies and immunological competition with gp120; and

(e) selecting and purifying said monoclonal antibody produced by said hybridoma.

11. The method of claim 9 or 10, wherein said serum sample is HIV positive human sera.

12. The method of claim 9 or 10, wherein said serum sample is sera from a second mammalian host immunized with a gp120.

13. The method of claim 9 or 10, wherein said serum sample is sera from a second mammalian host immunized with a compound according to claim 1.

14. A method for generating polyclonal antibodies for use as a vaccine or in immunotherapy, comprising the steps of:

(a) immunizing a first mammalian host with an immunogenic formulation comprising a compound according to claim 1 or 2;

(b) applying a serum sample from said host to a solid support coated with a gp120, wherein a first plurality of antibodies in said sample complex with said gp120, separating said solid support from said serum sample, and eluting said first plurality of antibodies from said solid support;

(c) immunizing a second mammalian host with an immunogenic formulation comprising said first plurality of antibodies; and

(d) selecting and purifying a second plurality of polyclonal antibodies from the sera of said second host, wherein said second plurality of polyclonal antibodies are characterized by their immunological reactivity with said first plurality of antibodies and immunological competition with said gp120.

15. A method for generating monoclonal antibodies for use as a vaccine or in immunotherapy, comprising the steps of:

(a) immunizing a first mammalian host with an immunogenic formulation comprising a compound according to claim 1 or 2;

(b) applying a serum sample from said host to a solid support coated with a gp120, wherein a first plurality of antibodies in said sample complex with said gp120, separating said solid support from said serum sample, and eluting said first plurality of antibodies from said solid support;

(c) immunizing a second mammalian host with an immunogenic formulation comprising said first plurality of antibodies;

(d) generating a hybridoma from an antibody producing organ of said second host, wherein said hybridoma is capable of producing a monoclonal antibody characterized by its immunological reactivity with said first plurality of antibodies and immunological competition with gp120; and

(e) selecting and purifying said monoclonal antibody produced by said hybridoma.

16. A method for generating polyclonal antibodies for use as a vaccine or in immunotherapy, comprising the steps of:

(a) immunizing a first mammalian host with an immunogenic formulation comprising a compound according to claim 1 or 2;

(b) generating a hybridoma from an antibody producing organ of said first host, wherein said hybridoma is capable of producing a first monoclonal antibody characterized by its immunological reactivity with said compound and immunological reactivity with gp120;

(c) immunizing a second mammalian host with an immunogenic formulation comprising said first monoclonal antibody; and

(d) selecting and purifying a plurality of polyclonal antibodies from the sera of said second host, wherein said polyclonal antibodies are characterized by their immunological reactivity with said first monoclonal antibody and immunological competition with said gp120.

17. A method for generating a monoclonal antibody for use as a vaccine or in immunotherapy, comprising the steps of:

(a) immunizing a first mammalian host with an immunogenic formulation comprising a compound according to claim 1 or 2;

(b) generating a hybridoma from an antibody producing organ of said first host, wherein said hybridoma is capable of producing a first monoclonal antibody characterized by its immunological reactivity with said compound and immunological reactivity with gp120;

(c) immunizing a second mammalian host with an immunogenic formulation comprising said first monoclonal antibody;

(d) generating a hybridoma from an antibody producing organ of said second host, wherein said hybridoma is capable of producing a second monoclonal antibody characterized by its immunological reactivity with said first monoclonal antibody and immunological competition with gp120; and

(e) selecting and purifying said second monoclonal antibody produced by said hybridoma.

18. Polyclonal antibodies generated according to the method of claim 9, 14, or 16.

19. A monoclonal antibody generated according to the method of claim 10, 15, or 17.

20. A monoclonal antibody that is immunologically reactive with the compound of claim 1 or 2.

21. An immunoassay method for the detection of anti-HIV antibodies comprising:

(a) coating a solid support with an effective amount of a compound according to claim 1 or 2 as an antigen;

(b) adding a test sera diluted with a buffer, wherein anti-HIV antibodies in the test sera form a compound/antibody complex with said compound;

(c) incubating the mixture at room temperature; and

(d) detecting the presence of said antibody/compound complex.

22. An immunoassay method according to claim 21 wherein step (d) comprises introducing a second known antibody capable of complexing with said anti-HIV antibody, wherein said second antibody is labeled with an enzyme, and adding a substrate which reacts with the enzyme to form a colored product.

23. An immunoassay method according to claim 21 wherein step (d) comprises introducing a second known antibody capable of complexing with said anti-HIV antibody, wherein said second antibody is labeled with a radioactive element.

24. An immunoassay method according to claim 21 wherein the compound/antibody complex is detectable as an agglutination.

25. A test kit for the detection of anti-HIV antibodies comprising:

(a) a solid support; and

(b) an immunoabsorbant comprising a compound according to claim 1 or 2, coated onto said solid support.

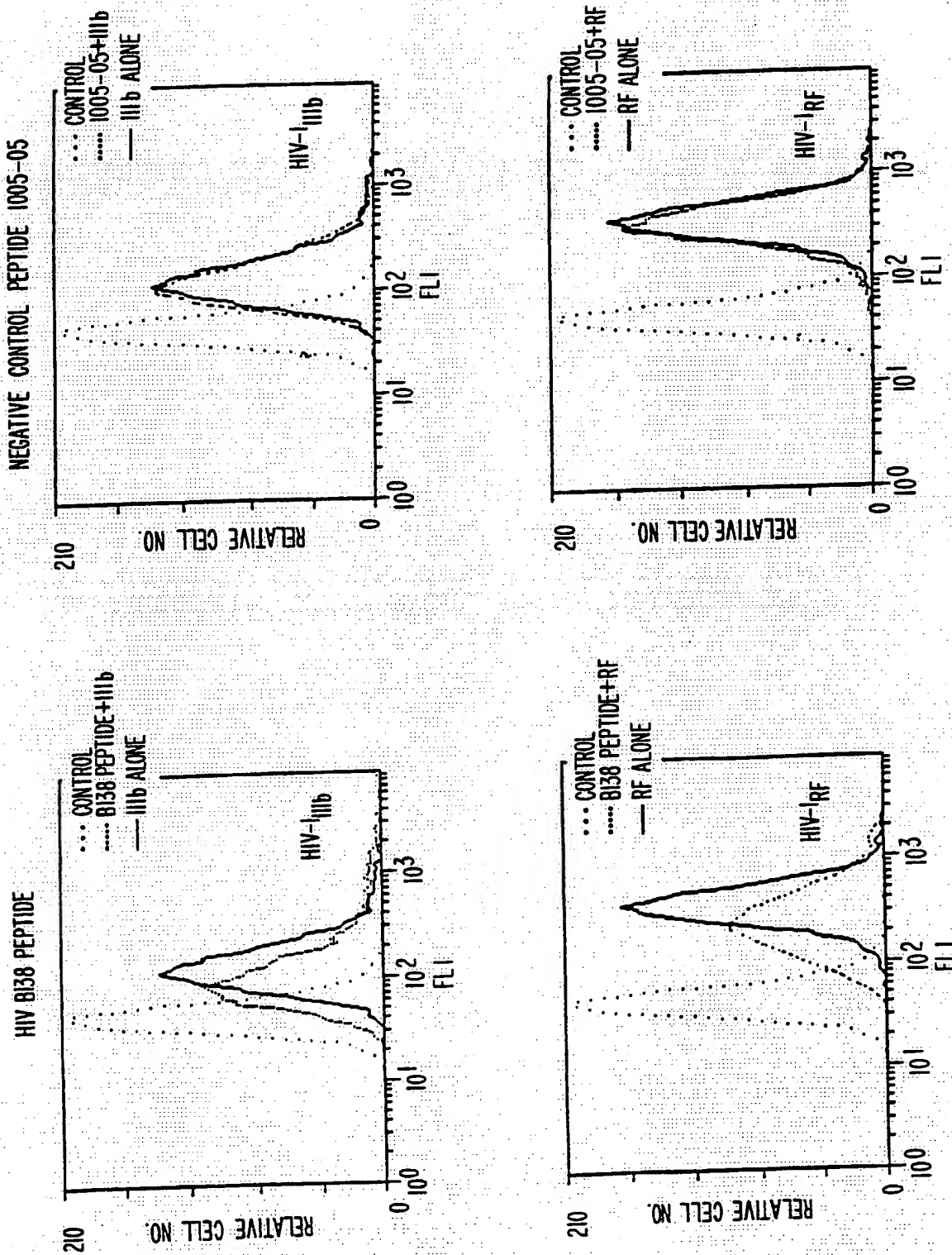
SUBSTITUTE SHEET

PEPTIDE NUMBER	SEQUENCE	ANTISYNCYTIAL POTENCY (μg/ml) IIIB	SF33	HIV-2	LAV-1
	418 445				
1005/45 (gp120)	C R I K Q F I N M W Q E V G K A M Y A P P I S G Q I R C	200 μg+	200 μg−	200 μg−	200 μg+
B26	R I K Q F I N M W Q E V G K A M Y A P P I S G Q I R C	200 μg+			
B119	C R I K Q F I N M W Q E V G K A M Y A P P	200 μg+			
1209/16	K Q F I N M W Q E V G K A M Y A P P I S G Q I R	440 μg+			
B138	K Q F I N M W Q E V G K A M Y A P P	40 μg+	40 μg+	20 μg+	40 μg+
1005/34	K Q I I N M W Q K V G K A M Y A P P	100 μg+	100 μg+	100 μg+	100 μg+
B92	K Q F I N I W L E W Y K A M Y A P P	400 μg−			
1029/04	R Q I I N T W H K V G K N V Y L P P	200 μg−	200 μg−	200 μg−	200 μg−
A24	W Q E V G K A M Y A P P	150 μg+			
A2A	K A M Y A P P	1 mg−			
	410 438				
1017/08 (gp120)	G D S T I T L P C R I K Q F I N M W Q E V G K A M Y A P P	400 μg+			
1005/33	G D S T I T L P C R I K Q F I N M W Q E	200 μg+			
	437 459				
1017/88 (gp120)	P P I S G Q I R C S S N I T G L L L T R D G G	200 μg+			
A5a	S N I T G L L L T R	300 μg−			
	473 483				
A27 (gp120)	F R P G G G D M R D N W R S E L	300 μg−			
A26	G D M R D N W R S E L	200 μg−			
1017/51 (Malaria)	D I E K K I A K M E K A S S V E N V Y N S	400 μg−			
	72 83				
1005/03 (MHC)	E E Y V R F D S D V G E	400 μg−			
	38 65				
1005/78 (CD4)	G N Q G S F L T K G P S K L N D R A D S R R S L W D Q G	400 μg−			

FIG. 1.

5/11

FIG. 2.


NEGATIVE CONTROL PEPTIDE 1005-05
HIV B138 PEPTIDE
··· CONTROL
···· 1005-05+IIIb
—— IIIb ALONE
···· B138 PEPTIDE+IIIb
HIV-1IIIb
··· CONTROL
···· 1005-05+RF
—— RF ALONE
···· B138 PEPTIDE+RF
HIV-1RF
RELATIVE CELL NO.
210
0
100
101
102
103
FL1

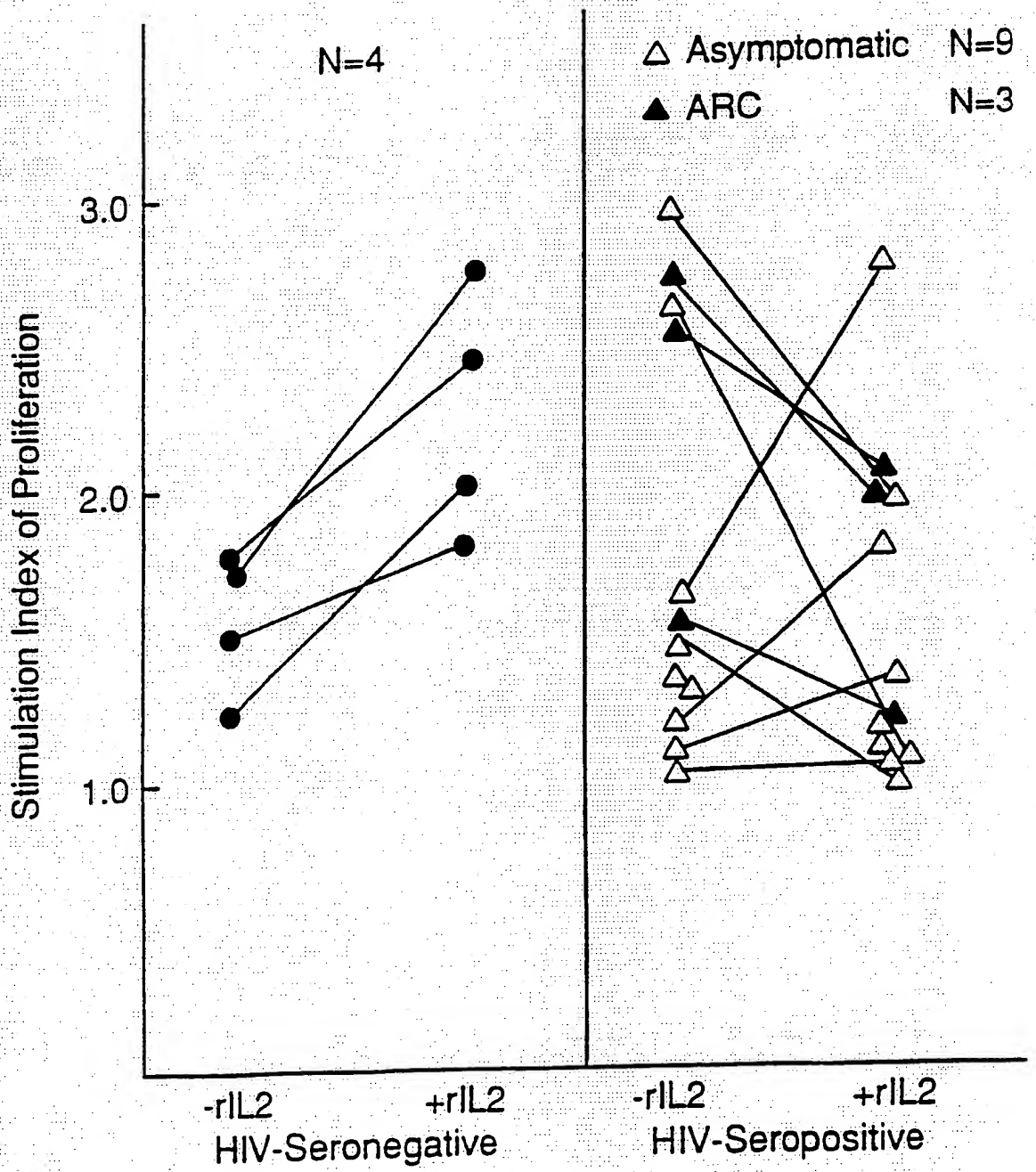
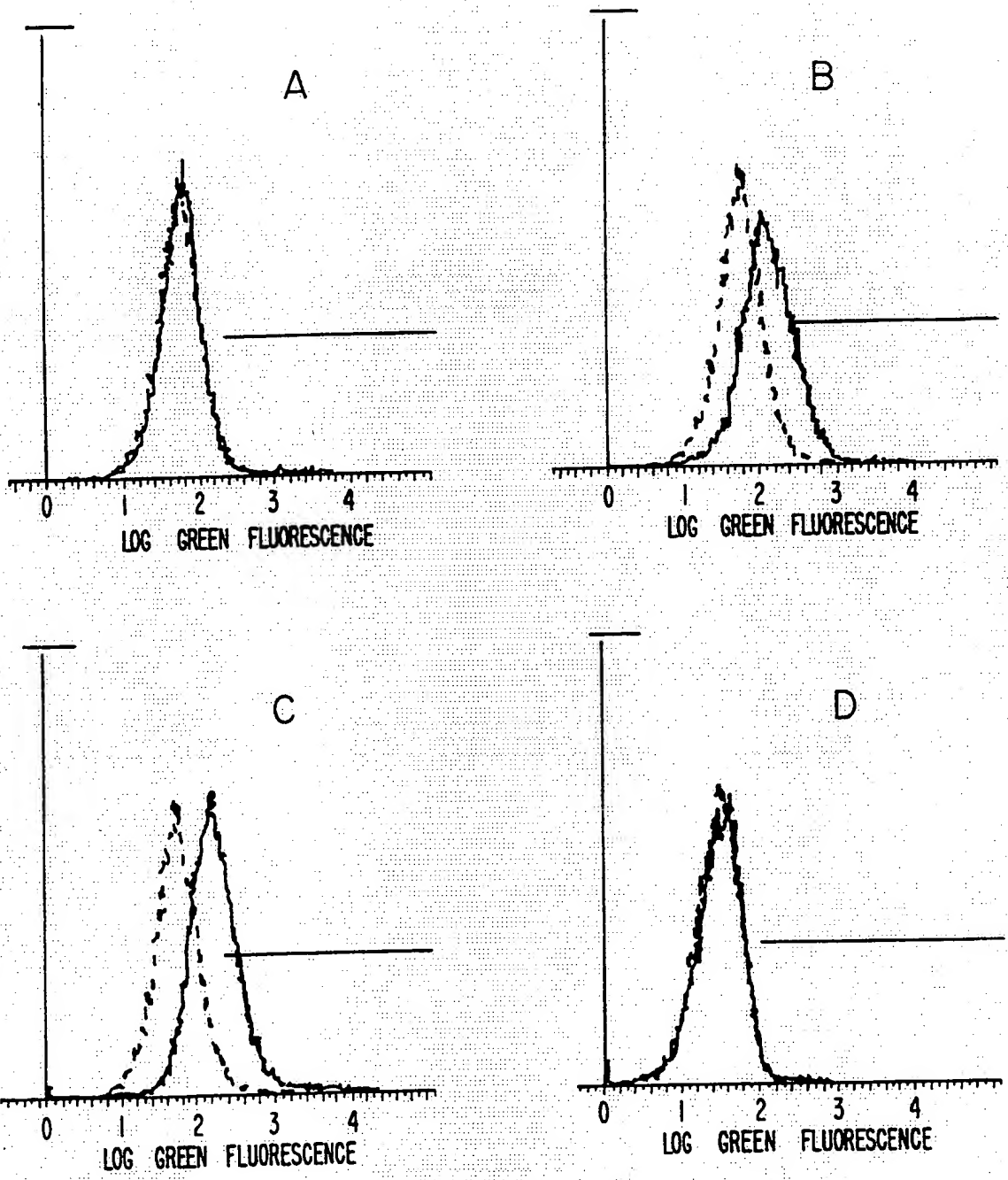


FIG 3.

SUBSTITUTE SHEET



A-H9/IIIb + NMS

B-H9/IIIb ± αδρ120 DBW

C-H9/IIIb + αβ138

D-H9 + αβ138

FIG. 4.

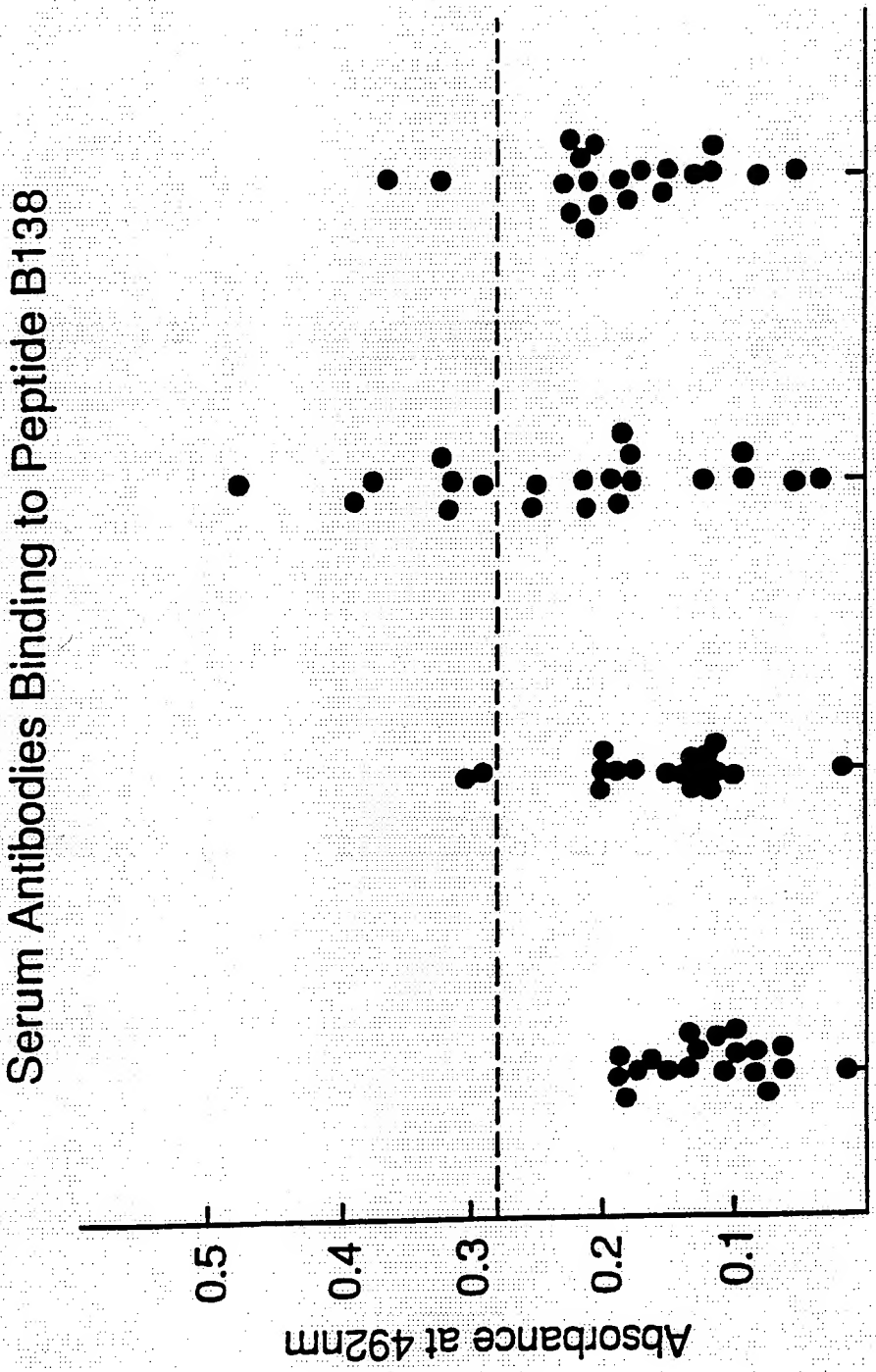

Serum Antibodies Binding to Peptide B138
Absorbance at 492nm
0.5
0.4
0.3
0.2
0.1
HIV-
Asymptomatic
N=19
HIV+
Asymptomatic
N=18
HIV+
ARC
N=21
HIV+
AIDS
N=20

FIG. 5.